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Transcriptional selectivity in early mouse embryos: a qualitative study

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ABSTRACT

The mouse zygotic genome is activated at the 2-cell stage. At this stage, microinjected DNA can be expressed and its transcription, analysed qualitatively with LacZ reporter genes, has the following characteristics (i) Sp1-sensitive promoters are active; (ii) the SV40 early promoter does not require upstream enhancers; (iii) genes driven by the −447, +563 region of murine leukemia virus (M-MuLV) are repressed and; (iv) activation of promoters is possible as shown for the promoter of acetylcholine receptor α-subunit by MyoD. This transactivation can occur before the formation of the zygotic genome. The transcriptional selectivity of 2-cell embryos also characterizes oocytes and 4-cell embryos. Therefore the elements involved are present in the oocytes and they persist after fertilization. This transcriptional selectivity has numerous common characteristics with that in EC cells, and may be indicative of a genetic control program specific for multipotential cells.

INTRODUCTION

Problems related to transcription specificity are central to the understanding of development. The specification of cell fate during embryogenesis is determined by complex interactions of classes of transcriptional regulators organized in networks. The hierarchical action of the maternal, gap, pair rule, segment polarity and homeotic gene classes in Drosophila are examples of such organization (1, 2, 3). The picture which emerges from the analysis of these systems and from the analysis of C. elegans (4) remains, however, completely speculative when applied to the early development of mammals. Our poor knowledge of these questions in mammals is due to the paucity of mutants affecting development (5) and to the difficulties in obtaining large quantities of material for biochemical analysis.

Recently some of these technical problems have been overcome. For instance, it is now possible to study gene expression at the cellular level using reporter molecules (6, 7, 8 , 9). This is an important advance as changes in the pattern of gene expression may indicate changes in the program of control of gene expression. If the change is due to the synthesis of new transcriptional regulators, further studies may give access to a network of more fundamental control molecules. Indeed, it is now possible to isolate a transcriptional regulator by its binding to specific nucleotide sequences and to isolate the complementary cDNA gene coding for the regulator following the determination of part of its amino-acid sequence. The study of the control of the regulator may then give access to the presumed network of molecules which control it. Therefore, the identification of any gene whose expression is developmentally controlled could be helpful for molecular studies of embryogenesis. One approach towards the identification of such regulated genes is by gene-trap experiments using reporter constructs subject to position-effect (10, 11, 12). Another approach is to test promoter function of available sequences at various given stages of embryogenesis with appropriately designed reporter constructs (7, 9, 13, 14, 15, 16).

For the reasons mentioned above, we have initiated a description of the pattern of gene expression in pre-implantation embryos using LacZ constructs. The promoters analyzed in this study were selected for their differing activities in EC cells and differentiated cells. EC cells have many phenotypic characteristics that are shared by normal embryo cells (17, 18) and therefore certain of their biochemical characteristics may be shared by their in vivo counterparts. In EC cells, the expression of the genomes of DNA tumor viruses (SV40 and Polyoma) and of murine retroviruses is impaired. The mechanisms of blockage of these viruses have been extensively analysed expression of SV40 is impaired by a mechanism which involves the control of transcription (19, 20, 21). In addition, it has been proposed that the undifferentiated cells contain a trans-acting regulatory factor that reduces transcription by interacting with the SV40 enhancers (20, 22); expression of Polyoma is impaired by the non functionality of its enhancer region (23, 24, 25, 26); and expression of M-MuLV is blocked in part because of the absence of function of the viral long terminal repeat promoter (20, 27) and in part because of the presence of an EC-specific repressor binding site element which has been mapped downstream of the transcriptional start site (28 and references therein). Other differentially regulated genes in EC cells include the genes of

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the H-2 complex (reviewed in 29). Their inactivity in EC cells has been attributed to the absence of the major specific activator of this family of genes, KBF1 (30). In addition, it has been shown that the A enhancer region acts as a negative control element in F9 cells (31).

This paper describes the expression of the LacZ gene driven by elements of the promoter regions of the genes described above and in addition of genes ubiquitously expressed in somatic cells but activated at different stages of embryogenesis.

MATERIALS AND METHODS

Plasmid construction

pM-MuLV-SV LacZ is described in (6); pMMuLVnlsLacZ, pM-MuLV-SV nlsLacZ, pM-MuLV-β-act LacZ are described in (7); pM-MuLV-AChR nlsLacZΔEnh was derived from pM-MuLV nlsLacZ ΔEnh by inserting the 3.3kb fragment of 3.3 CAT (32) containing the promoter of the alpha subunit of the receptor of acetylcholine into the SalI site. The enhancers of the LTR3' absent from pM-MuLV nlsLacZΔEnh were deleted as described in (27); pM-MuLV nlsLacZΔEnh was linearized with SalI and treated with Klenow enzyme. The linear DNA was ligated to the 1.7kb EcoRI-Xma III fragment of HPRT promoter (gift from Dr Friedmann, 33) resulting in pM-MuLV-HPRT nlsLacZΔEnh. pM-MuLV-IL-2 LacZΔEnh was from Dr Emilie, and carries the promoter on 2 kb HindIII-PstI fragment (34). pSEG5 (35), was digested with SalI and Bam HI, treated with Klenow enzyme and religated, thereby deleting the six GC boxes, and the construct called pΔGC. The 0.3 kb EcoRI-SfiI fragment of pA56, pSVS1 (22, 36), pΔGC and pSEG5, were each ligated to the 5.6 kb SfiI-PstI fragment of pM-MuLV-SV nlsLacZ ΔEnh and the PstI-EcoRI fragment of pGEM2 to give pA56nlsLacZ, pSVS1nlsLacZ, pΔGCnlsLacZ and pSEG5nlsLacZ respectively (see Fig.1).The SalI-PstI fragment of pSEG5nlsLacZ was ligated to the SalI-PstI fragment of pGEM2 to give pTATAnlsLacZ.The 3.5 kb SalI-BamHI fragment of pMMuLVnlsLacZΔEnh was ligated to the SalI-BamHI fragment of pTZ18,1R. A 1kb BamHI fragment containing SV40 large T intron and the polyadenylation signal of SV 40 was inserted at the BamHI site of this construct and the 260bp Pvu II-Bgl II fragment from pAG0 (containing the tk promoter) treated with Klenow enzyme was inserted at the SalI site treated with Klenow enzyme to give ptknlsLacZA1. L7RHβ-gal was from 37. All constructions were performed using conventional methods and commercially available restriction enzymes.

Preparation of linearized DNA and religation

All DNA fragments were purified on glass beads. H-2K^{b}LacZ is a Hind III-EcoRI fragment of pH-2KbLacZ (gift from Dr Israël, 38) containing the promoter H-2K^b and the LacZ gene. Other inserts were obtained using restriction sites indicated in Fig. 1 and Fig. 3. For microinjection into oocytes, purified inserts were religated at 1.3 nM for 18 hr at 15°C. The efficiency of ligation was verified after separation on agarose gels.

Microinjection of fertilized eggs and preparation of oocytes

Fertilized eggs were obtained from the oviducts of (C57BL/6J×DBA2)F1 females mated with F1 males of the same strain. Manipulation of mice and eggs and microinjection were carried out as previously described (39, 40). Eggs were injected with 500 to 1,000 copies of the appropriate construct 12−14 hours post fertilization. Expression was analysed at various times thereafter.

Oocytes were dissected manually out of follicules of ovaries (41) from female (C57BL/6J×DBA2) F1 mice that were 13−14 days old. The oocytes were cultured in standard egg culture medium (42 and references therein). DNA (supercoiled or religated inserts) was injected into the germinal vesicle 1 to 4 hours after isolation.

Transfection of cells and detection of β-galactosidase

Transfection of cells with the CaPO4 precipitate method was performed as described in (43). β-galactosidase activity was detected in cells and eggs after fixation as previously described (6).

RESULTS

Background

Two reporter genes whose expression can be visualised in single cells were used nlsLacZ (7) which codes for a β-galactosidase fused to the nuclear localization signal peptide of SV40 large T antigen and which is therefore found associated with nuclei and

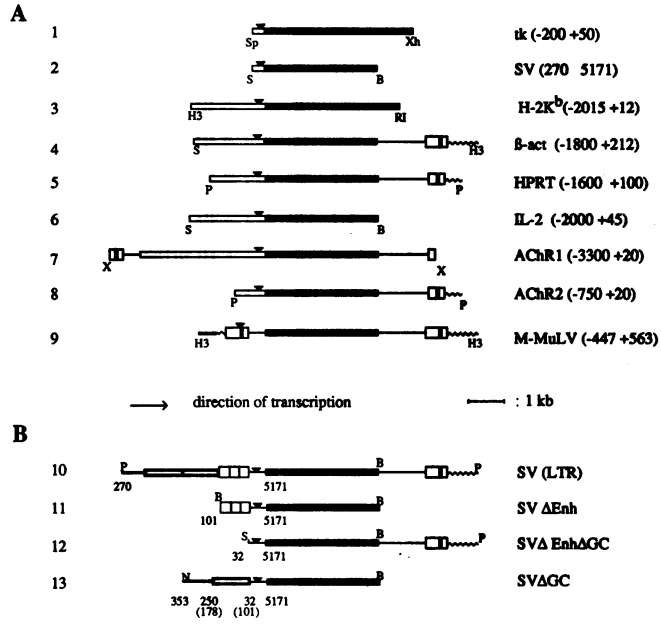

Figure 1. Structure of the DNA constructs. A), Schematic representation of the inserts used for microinjection in oocytes, 1-cell and 2-cell embryos. The elements are represented to scale. Opened boxes indicate the promotor. Black boxes indicate polyadenylation signals. The bacterial LacZ gene is shown as shaded boxes and MoMuLV untranslationed sequences as lines. Arrows represent the position of the initiation point of transcription. In parentheses, the nucleotide numbers reference the 5' and 3' ends of the fragment containing the promoter. Except for SV40 the sequences have been numbered with respect to the start point of transcription. Origin of the inserts is indicated in Materials and Methods. References are as follow tk (47); SV40 early (44); H-2Kb (38); β-act (57); HPRT (33); IL-2 (34); AChR (32); M-MuLV (45). Letters under the diagrams represent the restriction sites used to obtain the inserts (S SalI; B BamHI; H3 HindIII; P PstI; X XbaI; Sp SphI; Xh Xho I; N NcoI; RI EcoRI). B), Schematic representation of the inserts to test the activity of the SV40 early promoter. Same schematic representation than in (A) but, the promoter is not represented to scale. (10) linear insert containing a complete SV40 promoter. (11) linear insert containing the three 21 pb repeats and the TATA box of SV40 (derived from pSEG nlsLacZ). (12) linear insert containing the TATA box of SV40 (derived from pSEG nlsLacZ). (13) linear insert deleted for the 21 pb repeats but containing a complete enhancer region.

LacZ which codes for cytoplasmic β-galactosidase (6). These reporter genes were positioned downstream of promoters of different origins (Fig. 1) and the constructs were microinjected into either oocytes, fertilized eggs or one blastomere of 2-cell embryos. The sensitivity of the detection method (histochemical staining using X-gal as a substrate of β-galactosidase) (6) was assessed by microinjecting purified β-galactosidase into fertilized eggs or into one blastomere of 2-cell embryos. A detectable signal was obtained for the injections of 10^4 molecules of enzyme. No background activity was detected in control eggs.

No attempt was made to relate the percentage of β-galactosidase positive embryos to transcriptional efficiency. With the X-gal assay, constructs can clearly be grouped into two classes (i) constructs whose microinjection resulted in β-galactosidase positive embryos (from 15% to 60%) which were considered functional and (ii) constructs whose microinjection did not result in β-galactosidase positive embryos (less than 1%) which were considered ineffective. Usually at least 50 to 100 eggs were examined before admitting ineffectiveness.

All constructs were tested both as supercoiled DNA (plasmids) and as inserts deleted of plasmid DNA sequences, to unambiguously demonstrate that expression was due only to the eukaryotic sequences. As there was no discrepancy in the results between supercoiled DNA and linear inserts in 1-cell and 2-cell embryos (see below) we concluded that both forms of DNA are adequate substrates for transcription. In contrast, in oocytes linear DNA is inactive for transcription (13 and our unpublished results). Therefore before the injection the inserts were religated.

The number of copies injected into eggs or in the nuclei of one of the blastomeres of 2-cell embryos was 1000−3000 per egg (unless indicated) as eggs are large, this results in a low gene concentration. This condition was chosen to minimise titration effects especially of repressor-like molecules (see for instance 20).

The lag period for expression is about 3−5 hours after microinjection both in cells in culture and in early embryos (Vernet et al., in preparation). β-galactosidase production was measured 20 hours after injection of constructs. To check for expression in the 2-cell stage, late 1-cell embryos (24 hours post hCG injection) were injected into one of the pronuclei. To check for expression in the 4-cell stage (48 hours post hCG injection), the nuclei of one of the blastomeres of a 2-cell stage embryo was injected. Finally, in experiments to check for expression in oocytes, dbc-AMP, which may influence expression, was not used and only ovaries of 13−14 day-old female mice were used as a source of oocytes (46).

Only those microinjected eggs which underwent normal time course of cleavage are included in the results. Arrested eggs which did not reach the 2-cell stage (29% of microinjected eggs) or arrested 2-cell stage which did not reach the 4-cell stage (20% of microinjected 2-cell embryos) are therefore discarded.

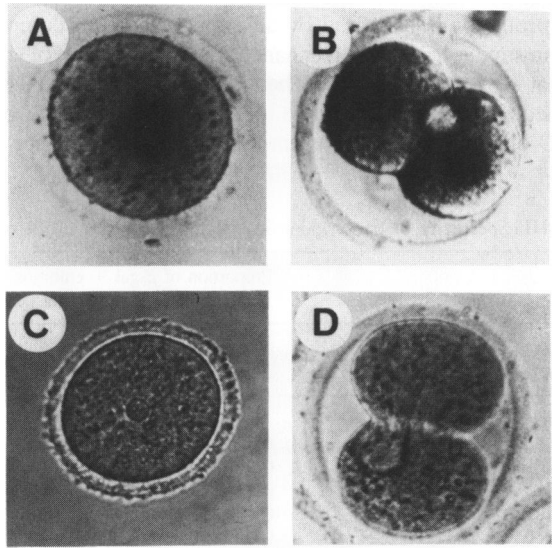

Figure 2. Expression of the nlsLacZ gene in oocytes and in 2-cell stage embryos. A, Oocyte, staining in the germinal vesicle. B, 2-cell embryo during interphase (the location of β-galactosidase is nuclear). Microinjection of the nlsLacZ construct was 20 hours before. Histochemical detection of β-galactosidase by X-gal staining. C, Oocyte, control. D, 2-cell embryo, control.

The pattern of promoter utilization in 2-cell embryos

Various promoters were analysed (Fig. 1). Expression was detected by X-gal staining (Fig. 2).

The promoter of thymidine kinase (tk): The regulation of the promoter of the tk gene of the herpes simplex virus (HSV) is relatively simple. The upstream region of tk consists of a TATA box and a single CAAT box (recognized by a transcription factor known as CTF) flanked by two GGGCGG hexanucleotides (named GC box in this article) (recognized by Sp1) 80 nucleotides upstream of the start site of transcription (reviewed in 47). These promoter elements are common to many genes transcribed by RNA Polymerase II and the deletion of any one of them seriously diminishes transcription activity. In addition it has been shown that Sp1 functions in concert with CTF to facilitate expression of the tk promoter (48). Not unexpectedly, the tk insert (Fig.1, Construct 1) was functional in 2-cell embryos (53% of embryos were positive, Table I).

SV40 early promoter: In comparison to the promoter of tk, the promoter of the early functions of SV40 is complex. It consists of an upstream region containing a TATA box and six tandemly arranged GC boxes (no CAAT box). In addition, this Sp1-responsive promoter needs to be enhanced by a number of transcription factors which bind sequence motifs spread over a region of about 200 bp (designated here the enhancer region) located at −101 to −290 (36, 49, 50, reviewed in 48).

In embryonal carcinoma cells, an activity of the SV 40 enhancer region has been described in transient assays but the level of

Table I. Specificity of transcription in 2-cell embryos analysed with linear DNA inserts and supercoiled plasmids.

	proportion of β-gal+embryos total number of embryos in parentheses	
	Inserts	Plasmids
1. tk	0.53 (64)	nd
2. SV	0.15 (45)	0.11 (46)
3. H-2Kb	0.23 (71)	0.15 (112)
4. β-act	0.28 (85)	0.30 (77)
5. HPRT	0.49 (122	0.30 (206)
6. IL-2	0.00 (61)	0.00 (57)
7. AChR1	0.00 (94)	0.00 (15)
9. M-MuLV	0.00 (85)	0.00 (114)

In vivo fertilized eggs were microinjected with 500 to 1000 copies of DNA molecules and cultured for 20 hours (2-cell stage) before X-gal staining. Numbers before the promoter refer to the structure of linear inserts detailed in Fig. 1. The structure of the plasmids are detailed in Materials and Methods. nd not determined.

enhancement was much lower than in differentiated cells (22, 21, 51) and the in vitro binding of nuclear proteins has been documented (52). In addition, in F9 cells the SV40 early promoter works efficiently in an enhancer-independent fashion (20, 22).

In 2-cell embryos the linear inserts SV (Fig 1, Construct 2) and SV(LTR) (Fig. 1, Construct 10) which contain the enhancer regions of the SV40 enhancer in addition to the upstream region were functional (Table I and Table II). Further to define the requirement of the transcription machinery in 2-cell embryos, the expression of inserts lacking different domains of the SV40 upstream region was studied (Fig. 1, Constructs 10 to 13). Deletion of the GC boxes (from nucleotide 32 to 101) resulted in inactive promoters in the presence (Fig. 1, SVΔGC, construct 13) or absence (Fig. 1, SVΔEnhΔGC, construct 12) of the SV40 enhancer region. However, surprisingly, the nlsLacZ gene was expressed from a linear insert lacking all enhancer elements (deletion from nucleotide 101, Fig. 1, SVDEnh, Construct 11) but carrying the GC boxes.

The promoter of the H-2K^b gene: The expression of transplantation antigens encoded by the K and D loci of the H-2 complex is developmentally regulated and in undifferentiated EC cells the antigens and their mRNA are undetectable. In the embryo they are not detected until embryonic day 10 at the mid-somite stage (53). The analysis of the H2K^b 5′ region has revealed two enhancer-like sequences, called A (at −193 to −158) and B (at −120 to −61). The enhancer A region interacts with four different factors KBF1 which act as a positive factor and is the major control element of the promoter and KBF2, AP2 and tumor necrosis-α responsive element (reviewed in 29). In undifferentiated EC cells, KBF1 is absent. It is induced when the cells are triggered to differentiate. In addition, the enhancer A region has been shown to act as a negative control element in F9 cells (31). The H-2K^b insert contains both the enhancer A and B regions of H-2K^b in addition to TATA and CAAT boxes. It was not expressed in PCC3 and PCC4 cells. However, in 2-cell embryos when linear inserts containing the same promoter, enhancers and the reporter gene were used (Fig. 1, Construct 3) expression was obtained (Table I).

Promoters of genes activated in pre-implantation embryos: β-actin mRNA is synthesized from the 2-cell stage, as demonstrated by measurements of the RNA content in pre-implantation embryos (54, 55, 56). It is believed to be constitutively active in all cells. In the rat promoter (57) a TATA box was identified at nucleotide −25 and a CAAT box at nucleotide −80. In β-act insert, a fragment of 1.7 kb is fused to the reporter gene LacZ (Fig. 1, Construct 4).

HPRT activity from the endogenous gene is observed from the 8-cell stage (58). The structure of the human HPRT locus has been determined (59). In contrast to β-actin it has a number of characteristics generally associated with housekeeping genes it lacks a conventional TATA and CAAT boxes and possesses an extremely GC-rich region and five GC boxes in the −30 −90 region. The mouse HPRT promoter has conserved these characteristics (60) and deletion analysis has showed that only 49 base pairs of the 5′ flanking sequence (including the first two GC boxes) is necessary for normal expression in cultured cells. In addition, a domain hypersensitive to DNaseI has been found within 200 bp of the translational start codon in both human HPRT (61) and mouse HPRT (62). In HPRT insert, the 1.5kb fragment of the promoter region is fused to the reporter gene nlsLacZ (Fig.1, Construct 5).

Despite the difference in the timing of activation of the endogenous β-actin and HPRT genes, both β-act and HPRT inserts were highly active in 2-cell embryos (Table I).

Promoters of tissue-specific genes IL-2 gene expression is regulated by a transcriptional enhancer lying between −319 and −52 bp from the initiation site (reviewed in 63). The functional properties of the enhancer are the following it is activated only in T cells through the antigen receptor and activation of protein kinase C. Two regions are of particular importance for its activation, that is the −285 to −255 (which binds NFAT-1) and −93 to −63 (which binds NFIL-2A). The ubiquitous Oct1 transcription factor (64) and NFKB which is released from an inactive form (NFKB/IKB) by protein kinase C following T-cell activation are also involved in IL-2 gene activation. The promoter of IL-2 includes a TATA but no CAAT or GC boxes. There is no Sp1 binding site.

The second tissue-specific gene promoter chosen for this study is from the acetylcholine receptor a-subunit (AChR-α). A functional TATA box at −20, a Sp1 binding site (at −50), and a CAAT box (at −75) have been found within a 200 bp segment. This promoter is activated by an enhancer which comprises a domain similar to the SV40 enhancer core and two MyoD binding sites (at −85 and −100)(65).The mutation of both MyoD binding sites led to an almost complete loss of activation of the gene by MyoD or myogenin in transient assays (65). The choice of this

Table II. Requirement of the GC boxes for the activity of the SV40 early promoter in 1-cell and 2-cell embryos.

	Proportion of β-gal+ embryos Total number of embryos (in parentheses)	
Injection Staining	1-cell stage 2-cell stage	1-cell stage 4-cell stage
Promoter		
10. SV (LTR)	0.21 (389)	0.23 (48)
11. SVΔEnh	0.34 (61)	0.36 (11)
12. SVΔEnhΔGC	0.00 (14)	0.00 (20)
13. SVΔGC	0.00 (57)	0.00 (26)

In vivo fertilized eggs were microinjected with 500 to 1000 copies of DNA molecules and cultured for 20 hours (2-cell stage) or 44 hours (4-cell stage) before X-gal staining. Numbers before the promoter refer to the structure of linear inserts detailed in Fig. 1.

Table III. Specificity of transcription in oocytes, 1-cell and 2-cell embryos.

		Proportion of β-gal + embryos Total number of embryos in parentheses	
Injection Staining	Oocytes Oocytes	1-cell stage 2-cell stage	2-cell stage 4-cell stage
Promoter			
5 HPRT	0.31 (57)	0.46 (43)	0.67 (31)
11 SVΔEnh	0.28 (56)	0.34 (61)	0.38 (93)
3 H-2Kb	0.39 (66)	0.23 (71)	0.43 (32)
9 M-MuLV	0.00 (71)	0.00 (85)	0.00 (86)
7 AChR1	0.00 (62)	0.00 (94)	0.00 (54)

For all constructs 1000 to 3000 copies were injected. The column 'Oocytes' AChR and M-MuLV correspond respectively to supercoiled DNA pM-MuLV-AChR nlsLacZΔEnh and to supercoiled DNA pM-MuLV-nlsLacZ. HPRT, H-2Kb and SVΔEnh correspond to religated inserts 5, 3 and 11 of Fig. 1. The column '1-cell stage−2-cell stage' repeats data from Table I and II. Under the columns '1-cell stage' and '2-cell stage' data are from injections of inserts referenced in Figure 1.

promoter was dictated by its property of being transactivable by myogenic factors (see below).

Neither of these promoters of genes with specialized function (IL-2 and AChR, Fig.1, Constructs 6 and 7) were active in 2-cell embryos (Table I and II). They were active, as expected when tested in their specific cell types (66 and our unpublished results).

Murine Leukemia Virus: The expression of M-MuLV and MSV is tightly negatively controlled in embryonal carcinoma cells. Extensive analysis has established that the promoter which is composed of a TATA box at −25 and a CAAT box at −80 (no Sp1 binding site) does not function because of the presence of repressor sequences within the 82 bp repeats (20) and 5′ to them at −345 (67) and because of the lack of positive activators (20, 68, 69). In addition, an EC-specific repressor binding site (70, 71) has been mapped to nucleotides +147/+174 (28). This strong silencer can repress heterologous promoters from an upstream position in EC cells. To learn whether these results can be extended to the egg, M-MuLVnlsLacZ inserts (Fig. 1, Construct 9) were microinjected into 1-cell embryos. As expected (7) no β-galactosidase was detected in 2-cell embryos (Table I). Detailed deletion analysis (to be described elsewhere) established that the LTR non-function and the repression mediated by the +147/+174 region are both properties shared by the 2-cell embryos

The pattern of expression at the 2-cell stage is observed at the 4-cell stage and is also characteristic of oocytes

The pattern of expression of genes microinjected at the 1-cell stage and observed at the 2-cell stage is characterized by the expression of constitutive promoters, the utilization of the SV40 early promoter lacking the enhancer region, the utilization of the H-2K^b promoter, and the non-utilization of the M-MuLV and tissue-specific promoters. These functional properties cannot be explained by the presence or the absence of a single factor but most certainly are related to the network of control molecules present at this stage. However, it was unclear whether this pattern persists after the activation of the zygotic genome at the 2-cell stage (72). Blastomeres of 2-cell embryos were therefore microinjected with molecules which best identify the pattern of control (HPRT, SVΔEnh, H-2K^b, M-MuLV and AChR, see Fig. 1) and were stained with X-gal, 20 hours later at the 4-cell stage. We observed the same pattern of expression of genes as at the 2-cell stage (Table III).

Another fundamental question is whether the factors conferring this pattern are already present in the oocyte or result from alterations or acquisitions occuring at the 1-cell stage (induced by fertilization for instance). To distinguish between these possibilities, constructs were microinjected into the germinal vesicle of oocytes. The samples were cultured for 20 hours and stained with X-gal (Fig. 2A). The pattern of expression of these oocytes was similar to that of the 2-cell stage (Table III). This suggests that the elements involved in the pattern of expression persist from the oocyte to the 2-cell stage.

Table IV. Transactivation of AChR nlsLacZ by MyoD in 2-cell embryos and in aphidicolin treated 1-cell embryos.

	Proportion of β-gal+ embryos Total number of embryos in parentheses 2-cell stage	 1-cell stage Aphidicolin treated
AChR nlsLacZ	0.00 (94)	0.00 (47)
AChR nlsLacZ +SVMyoD (2000 copies)	0.08 (64)	–
AChR nlsLacZ +SVMyoD-1 (10000 copies)	0.12 (73)	0.17 (35)
AChR nlsLacZ +SVneo (10000 copies)	0.00 (54)	–

One of the pronuclei of in vivo fertilized eggs was injected with 2000 copies of AChR2 (Fig. 1, construct 8) and cultured for 20 hours before X-gal staining. In co-injection experiments with SVMyoD, 2000 or 10000 copies of SV MyoD was added. In co-injection experiments with SVneo, 10000 copies of SVneo was added. Aphidicolin was supplemented to the culture medium just after microinjection (24 hours post hCG).

Transactivation of promoter can occur before the formation of the zygotic genome

The results presented above define certain of the cis-acting sequences that regulate gene expression in 2-cell embryos. But they do not answer the question of whether transcriptional regulators (other than those required for the basal function of the promoter) can work in 2-cell embryos. We addressed this question directly by taking advantage of the fact that the 5′ region of the AChR-α gene contains two MyoD binding sites at −85 and −100 and both are essential for full activity (65). AChR inserts (Fig. 1, Construct 8) were microinjected with and without SVMyoD, an expression vector for MyoD. The AChR inserts alone were inactive. Controls (coinjection of AChR and of large amounts of SVneo) designed to test whether SVMyoD titrated out a repressor of AChR promoter were also negative. In contrast, the AChR inserts was transactivated by MyoD (Table IV). This observation applies not only for morphologically 2-cell stage embryos but also for morphologically 1-cell stage embryos (still with two pronuclei) arrested by culturing them in aphidicolin, an inhibitor of DNA polymerases (Table IV). This suggests that the regulators can function effectively before the formation of the zygotic nucleus.

DISCUSSION

The data presented in this article give qualitative indications of some of the properties of the transcription apparatus in oocytes and in early embryos. We have analysed the functionality of several promoters and enhancer regions selected for their properties in EC cells. A few conclusions and several questions emerge from this screening.

First, there are no particular restrictions on the utilization of promoter sequences in 2-cell embryos. Promoters constituted of TATA and GC boxes (SV40), of TATA and CAAT boxes (H-2K^b, β-actin) or of GC boxes alone (HPRT) can all function at the early stages of development although the presence of these elements is not always by itself sufficient for expression (AChR-α, M-MuLV). Second, there is no aberrant expression of tissue-specific genes. For instance, the promoter of the IL-2 gene whose expression is restricted to T cells, and of the AChR-α subunit gene whose expression is restricted to muscle cells are silent, presumably because of the lack of the appropriate, specific combination of transcription factors in early embryos. Third, promoters can be activated by transcripion factors, for instance, the promoter of the AChR-α subunit gene by MyoD (this report) or the promoter of the metallothionein gene by heavy metals (73, 16) and the adenovirus Ella promoter by an EIA-like activity (9).

More intriguing is the observation that the activity of the SV40 early promoter remains high when the region containing the enhancers is deleted although it falls to zero when the GC boxes are deleted (Table II). The difference of activity between the two mutants in early embryos may indicate, as suggested by De Pamphilis et al., that enhancers are required late during embryogenesis (15). However, activators can work in early embryos at least when they are functionning as promoters elements (this report and 9) and the activity of certain promoters in early embryos is unusually high (15). We suggest therefore that certain transcription factors usually present in limiting quantities and consequently requiring activators in order to be positioned with high efficiency on their target sequences are at such high concentrations at these early stages that synergistic effects with other transcriptional factors are masked. This hypothesis could also explained that the enhancerless SV40 early promoter is more active in undifferentiated F9 EC cells than in fibroblasts (20, 22). Promoters such as the promoter of the AChR-a subunit gene which uses activators that are in limiting concentration would remain transactivatable. Another explanation for these characteristics is that the structure acquired by the plasmid may present peculiarities reflecting the state of the chromatin in these cell types. However the persistence of the potentiality to express an enhancer-deleted SV40 promoter in oocytes (14 and this report) and in 2−4 cell embryos after the activation of the zygotic genome argues against this hypothesis.

The overall pattern of utilization of promoters used in this study was qualitatively the same in microinjected oocytes, in 1-cell embryos and in 2-cell embryos (Table III). This pattern is characterized by the functionality of promoters of genes constitutively expressed and of the H-2K^b promoter, by the inactivity of the promoters of tissue-specific genes and by the repression of M-MuLV-driven genes. In addition, these stages are characterized by the functionality of the SV40 early promoter lacking the enhancer region and by their ability to transactivate the adenovirus Ella promoter (9). These results are most easily explained by postulating that the combination of elements required for this pattern in eggs is already present in the oocyte with a biologically similar specificity and that it persists at least until the activation of the zygotic genome. An example of regulatory factors in early embryos which are expressed in oocytes has been described for two octamer-binding proteins, Oct4 and Oct5 (74). It should be noticed that this hypothesis does not exclude quantitative differences in the level of expression at these various stages. Indeed, important variations in the level of expression of tk Luciferase and of Ella Luciferase in oocytes, arrested 1-cell embryos and 2-cell to morulas embryos have been reported (9, 15).

This pattern is very reminiscent of but distinct from that of multipotential cells in culture (EC). Only in EC cells is the H-2K^b promoter not functional. We will present elsewhere data demonstrating that the repression of M-MuLV is due to two controls, one mediated by LTR sequences and the other mediated by the +147/+174 repression region and, therefore, involves the same cis elements as in EC cells (28). If we accept the idea that EC cells share similarities with the cells of the inner cell mass (17, 18), it may indicate that at some point after the four-cell stage the pattern of expression changes.

The discovery and characterization of genes differentialy expressed may constitute a first step in the study of the mechanisms of the control of development other steps will include (1) to identify the factors involved in the expression of the 5′ region of the H-2K^b-LacZ gene and (2) the isolation of the factors controlling the repression of M-MuLV. These studies may give access to control elements turned on at different stages during early development.

Among the promoters active in early embryos one is from a gene activated late in pre-implantation embryos (HPRT). However, it nevertheless drives expression of LacZ at the 2-cell stage. Obviously the reasons for the lack of activity of the endogenous HPRT is not the absence of the trans-acting factors required for transcription. One explanation would be that we did not include a repressor region in the LacZ construct used. However, this does not interpret another finding, that transgenic HPRTnlsLacZ mice also do not express LacZ in 2-cell embryos (12) and therefore are also negatively regulated at this stage. This suggests the possibility that the structure of the HPRT and HPRTnlsLacZ genes in the embryonic genome precludes their expression. We have similarly interpreted the delay observed between the acquisition of a competent state for transcription at the late 1-cell stage and the major activation of the genome at the late 2-cell stage (Vernet et al., in preparation) and it could also explain the lack of expression of the H-2K^b gene in contrast to H-2K^b insert.

Finally, the similarities of the properties of oocytes, early embryo and EC cells are worth noting. These similarities are very extensive they presumably involve, as indicated above, positive and negative regulators necessary to explain the described transcriptional selectivity, to which can be added the transcription factors Oct4 and Oct 5 transcribed in both oocytes and EC cells (74) and the common property of both EC and early embryos to express the F101 Polyoma mutant (15, 74, 18) and to transactivate the adenovirus Ella promoter (75, 9). It is generally accepted that EC cells closely resemble cells of the inner cell mass (referenced in 76). These new findings further suggest that a number of elements controlling gene expression are specifically shared by all these cell types. Therefore, it is possible that these elements are components of a genetic control program characteristic of multipotential cells.

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